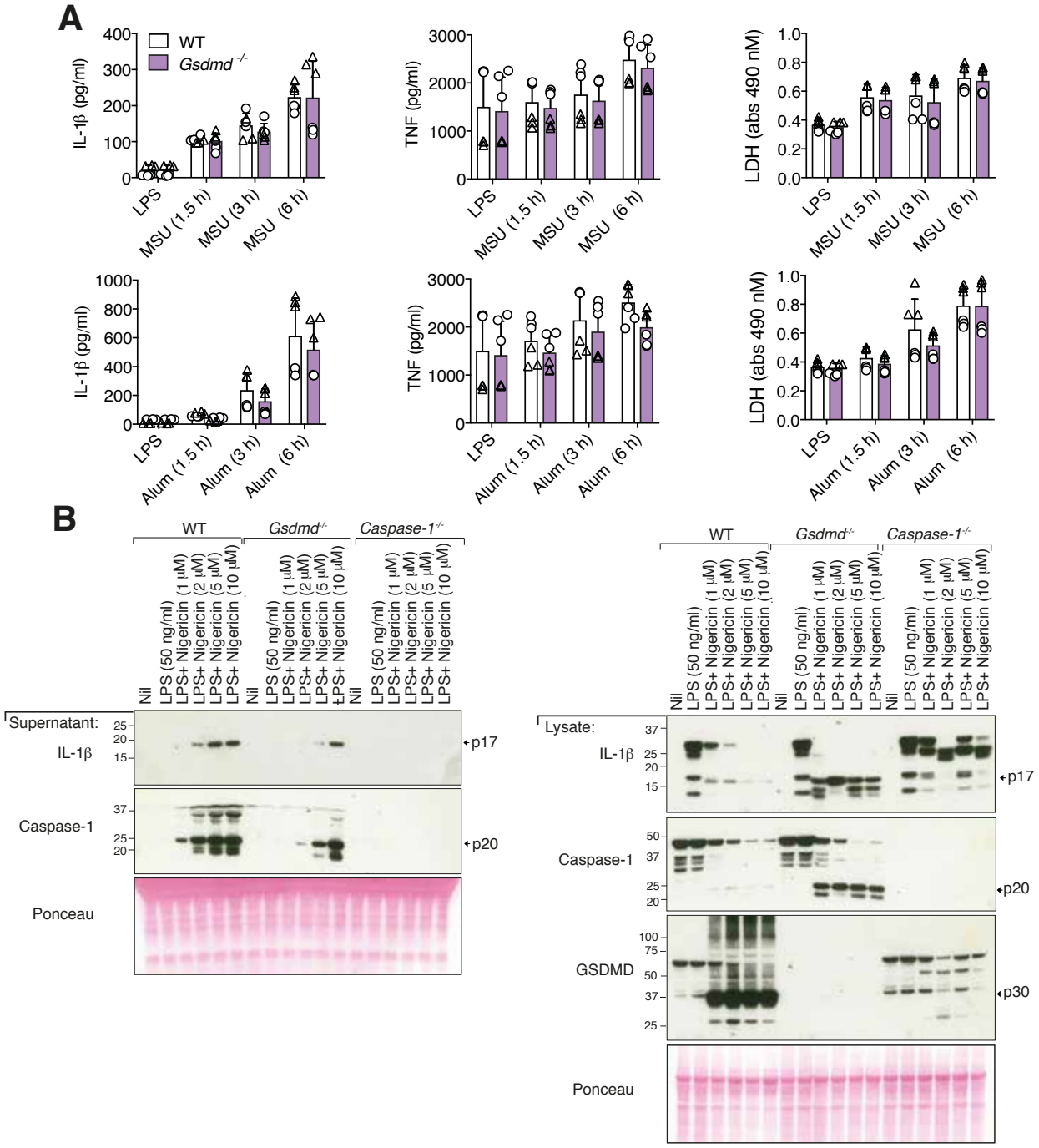
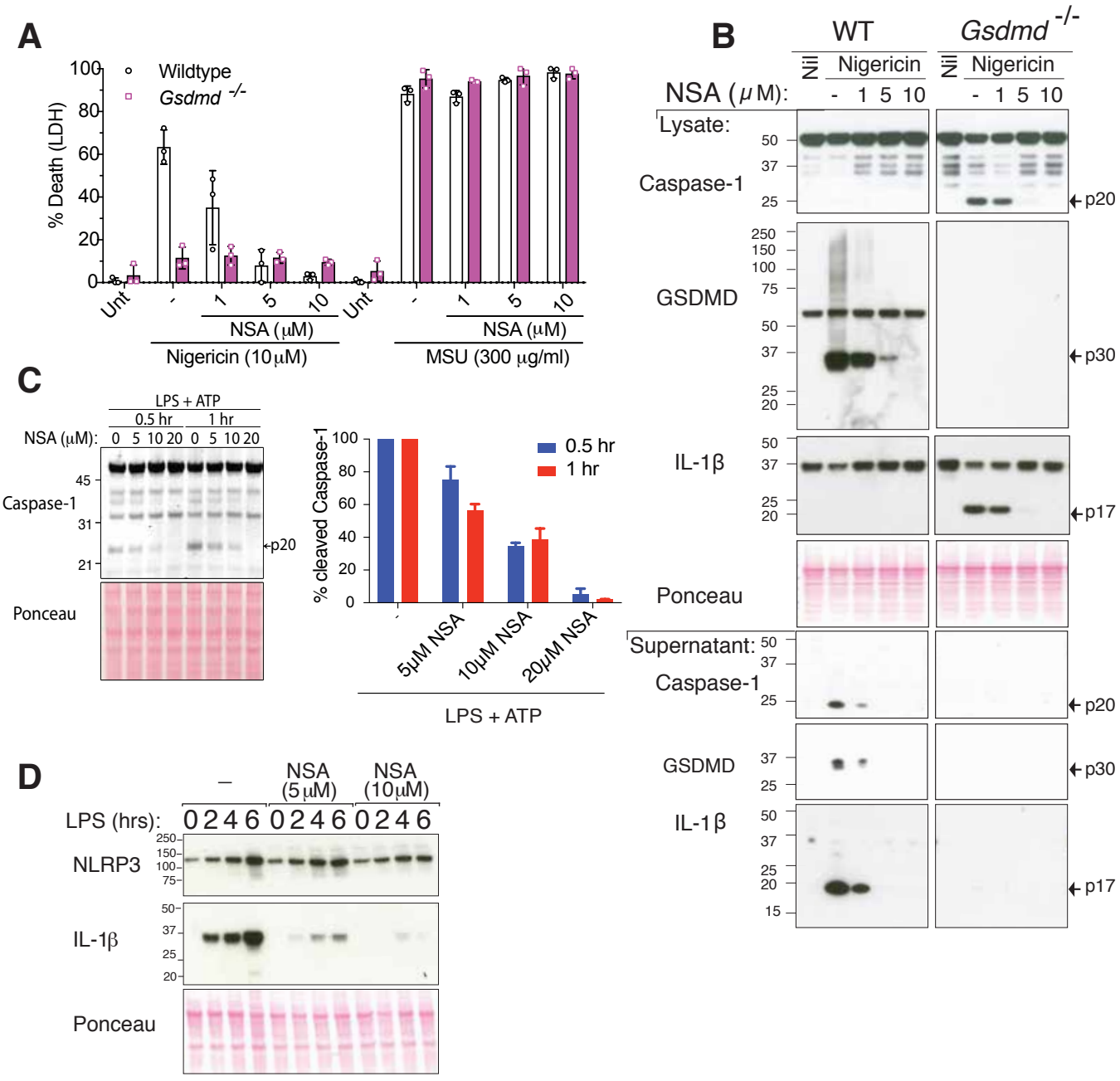
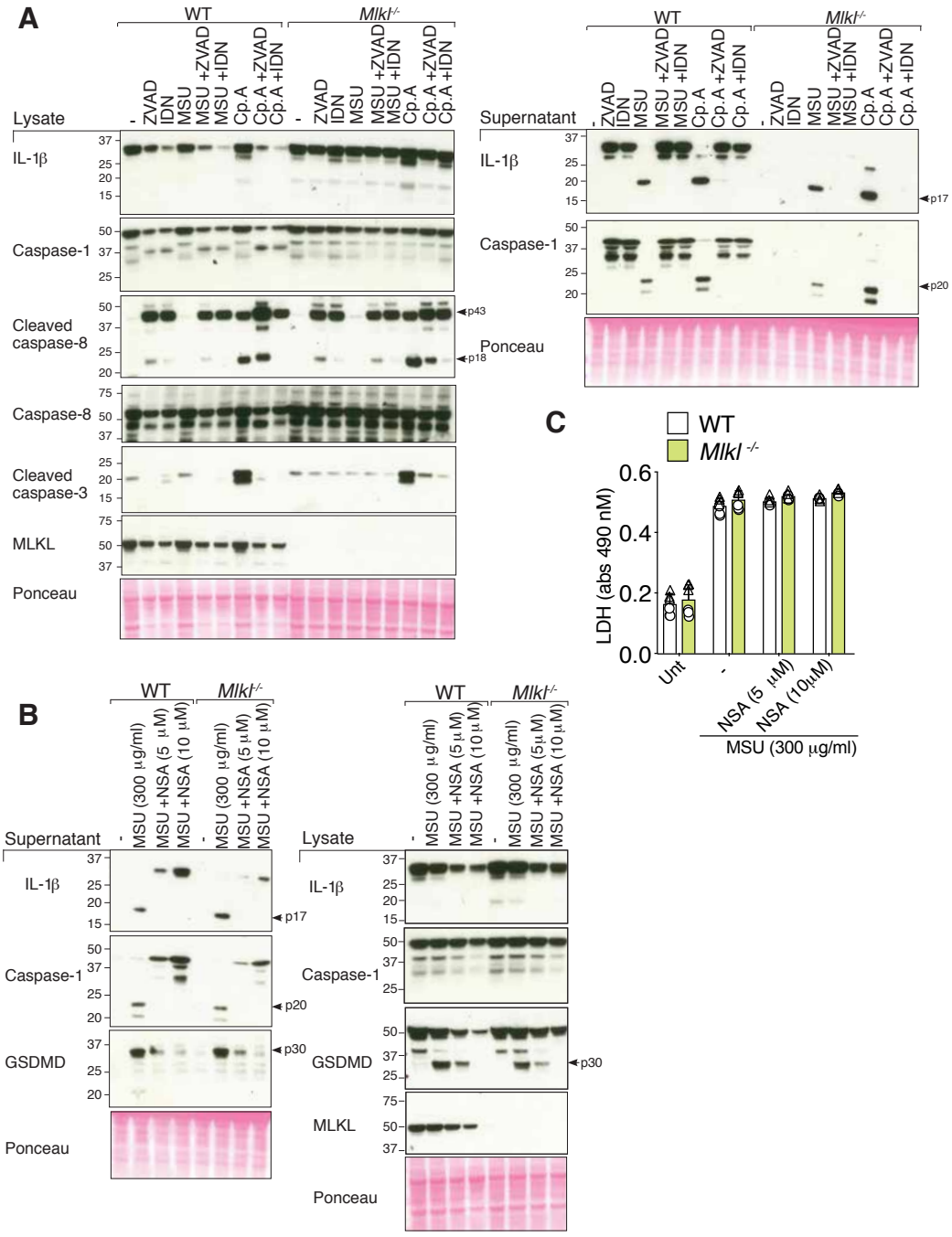


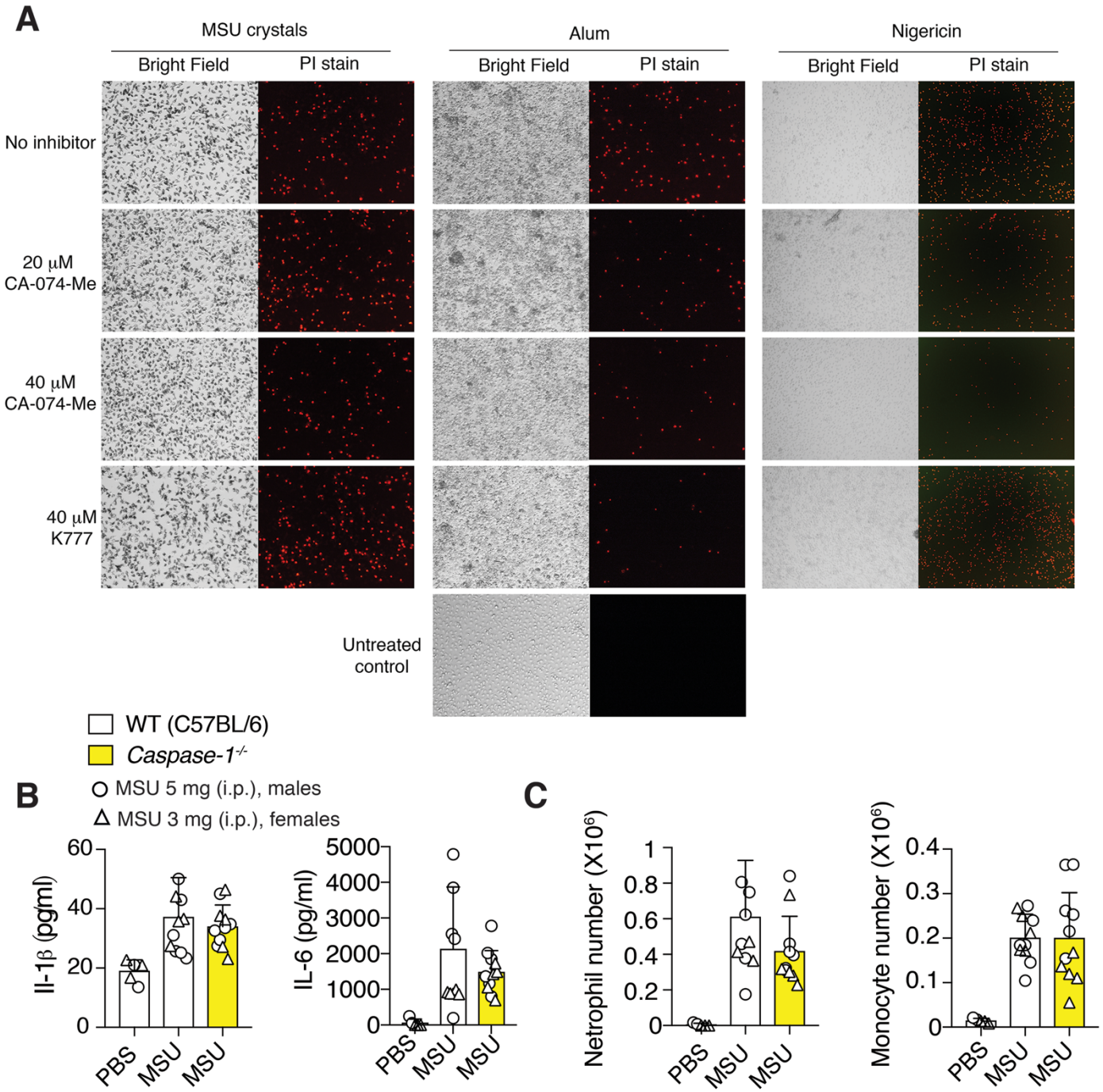
Supplementary Figure 1. GSDMD is dispensable for MSU crystal- and alum-mediated release of IL-1β. (**A**) IL-1β, TNF and LDH levels in the cell supernatant of BMDMs from WT (C57BL/6) and *Gsdmd*$^{-/-}$ mice that were primed with LPS (50 ng/ml, 2.5 hr) and then treated with MSU crystals (300 μg/ml) or alum (300 μg/ml) for the indicated times. (**B**) BMDMs from WT (C57BL/6), *Gsdmd*$^{-/-}$ and *Caspase-1*$^{-/-}$ mice were primed for 2.5 hr with LPS (50 ng/ml) and then treated with the indicated concentrations of nigericin (1 hr) and cell supernatant and total cell lysates analyzed by immunoblot. Ponceau staining indicates protein loading. (**A**) Mean ± SD of 5-6 replicates (symbols) pooled from two independent experiments. (**B**) Data are representative of two independent experiments.



Supplementary Figure 2. NSA inhibits the NLRP3 inflammasome upstream of GSDMD targeting. **(A)** BMDMs from WT and *Gsdmd*$^{-/-}$ mice primed for 2.5 hr with LPS (50 ng/ml), with the indicated concentrations of necrosulfonamide (NSA) added in the last 30 min of priming, were then treated with nigericin (10 μM, 1hr) or MSU crystals (300 μg/ml, 6hr) and LDH release assessed (% death calculated relative to LDH levels measured from detergent lysed cells). **(B)** BMDMs from WT and *Gsdmd*$^{-/-}$ mice were treated as described in (A) and cell supernatant and total cell lysates analysed by immunoblot. **(C)** RAW 264.7 macrophages expressing ASC were primed with LPS (100 ng/ml) for 4 hr, with the indicated concentrations of necrosulfonamide (NSA) added in the last 30 min of priming, then stimulated with ATP (5 mM) for the indicated times. Cell lysates and supernatants were combined and analyzed by immunoblot. Graph: the percentage of cleaved caspase-1 (p20) was quantified (normalized to LPS +ATP treated cells without NSA) by densitometry from 2 independent experiments. (**D**) BMDMs from WT mice were treated with the necrosulfonamide (NSA) for 30 min, then stimulated with LPS (50 ng/ml) for the indicated time points and total cell lysates analyzed by immunoblot. **(A to D)** Data are representative of 2-3 independent experiments.



Supplementary Figure 3. Figure 4. MLKL is not required for MSU crystal-induced death or NLRP3 inflammasome activation. (**A**) BMDMs from WT (C57BL/6) and *Mlkl*$^{-/-}$ mice primed with LPS (50 ng/ml, 2.5 hr), with Z-VAD-fmk (40 μM) or IDN-6556 (20 μM) added in the last 30 min of priming, were then treated with MSU crystals (300 μg/ml) or Smac-mimetic compound (Cp.A, 1 μM) for 6 hrs. Cell supernatants and total cell lysates were analyzed by immunoblot. (**B**) BMDMs from WT (C57BL/6) and *Mlkl*$^{-/-}$ mice primed with LPS (50 ng/ml, 2.5 hr), with the indicated concentrations of necrosulfonamide added in the last 30 min of priming, were then treated with MSU crystals (300 μg/ml) for 6 hrs. Cell supernatants and total cell lysates were analyzed by immunoblot, as indicated. Ponceau staining depicts protein loading. (**C**) LDH levels in the cell supernatant of BMDMs from WT (C57BL/6) and *Mlkl*$^{-/-}$ mice treated as described in (B). Western blot data are representative of two independent experiments. (C) Mean ± SD of 6 replicates pooled from two independent experiments.



Supplementary Figure 4. Impact of cathepsin inhibition or caspase-1 loss on MSU crystal-driven responses. (A) BMDMs pre-treated with the indicated cathepsin inhibitors for 40-60 min were then stimulated with MSU (300 μg/ml), alum (300 μg/ml) or nigericin (10 μM) for 4-5 hrs, stained with propidium iodide (PI), and imaged. PI uptake was quantified using Fiji software (see Figure 6A). **(B and C)** C57BL/6 and *Caspase-1*$^{-/-}$ mice were injected i.p. with carrier (PBS) or MSU crystals (3 mg-female mice [triangles] or 5 mg-male mice [circles]) and **(B)** IL-1β and IL-6 levels measured in the peritoneal lavage fluid supernatant after 4 hr. **(C)** Quantification of peritoneal influx of neutrophils and monocytes in C57BL/6 and *Caspase-1*$^{-/-}$ mice i.p. injected with carrier (PBS) or MSU crystals (3 mg -female mice[triangles] or 5 mg-male mice [circles]) for 4 hr. (**B and C**) Mean ± SD, n=10-11 mice per group (symbols) pooled from two independent experiments. In one experiment (circles) data showing WT mice injected with PBS or MSU are the same control WT mice depicted in Figure 7 (this experiment, comparing WT, *Caspase-1*$^{-/-}$ and *Gsdmd*$^{-/-}$ animals, was performed at the same time).